The Effect of Tadoples (*Rana catesbeiana*) Serum on Total and Differential Leukocyte in Rats (*Rattus norvegicus*): That Have Been Induced With Dimetylbenz-α-anthracene as Animal MODEL of Skin Cancer

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Keywords: Total leukocyte, differential leukocyte , *Rana catesbeiana*, serum, skin cancer.

Abstract: This study aimed to determine the effect of tadpoles (Rana catesbeiana) serum on total and differential leukocyte in animal model of white rat (Rattus norvegicus) that has been induced with skin cancer by Dimethylbenz-α-anthracene (DMBA). Male rats were induced by DMBA 20 mg/rat twice a week for 18 days to induce skin cancer. Tadpole's serum was injected intracutaneously after cancer had been known. Negative control (K-) was not induced with DMBA and tadpole's serum, while positive control grup (K+) was induced to DMBA. The treatment groups P1, P2, and P3 were induced with DMBA and injected tadpole's serum 100%; 75%; 25%/rat/day. This study used a Completely Randomized Design (CRD). Data were analyzed with ANOVA and continued by Duncan multiple test. The results obtained average number ± SD of total leukocyte K-, K+, P1, P2, and P3 were 12000.00±3814.88,3975.00±2451.36,8650.00± 5470.83, 6390.50 ± 3007.18 and 5590.00 ± 1292.18 respectively. There are significant differences regarding an increase in number of total leukocyte on treatment, but there is not real difference between K+, P1, P2 and P3. The results shsowed average number of limphocyte and monocyte are not significant but it is signiffant in granulocyte. Based on the results, it can be concluded that tadpoles serum is effective to increase number of total leukocyte and differential leukocyte (especially in granulocyte) in animal model of rats induced skin cancer.

1 INTRODUCTION

The prevalence of cancer in the population of all ages in Indonesia in 2003 reached 0.14 percent of the total population or 347,792 people (Ministry of Health, 2015). According to Indonesian Nutrition Network (2005), in Indonesia, cancer patients reached 6% of the population and deaths from this malignancy ranked second after death from infection. Based on a research conducted by Dhaygude (2006) in Mumbai, India during the period from January 2001 to December 2005, from 124 dogs who were autopsied or biopsy, the most recorded skin tumor was found in 74 (59.67%) followed by mammary gland tumor as many as 43

Cahyo, N., Wibawati, P., Primarizky, H., Prastiya, R., Purnama, M. and Damayanti, R.
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DOI: 10.5220/0007546905560560
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 556-560
ISBN: 978-989-758-348-3

tails (34,67%) and subsequently transmissible granuloma in genitals as many as 3 tails and ovarian and testicular tumor were 2 each (1.6%).

According to Mondou and Kaltenbach (1979), the tadpoles serum (*Rana catesbeiana*) contains thyroxine hormones that can enhance cellular immune responses. Thyroxine content in tadpole serum is 9.4 ng / ml, while thyroxine needed as therapy to increase immune is 0.01 - 0.1 microgram / ml. Leukocytes have a very important role in the body's defense, so the examination of the number of leukocytes is to support the diagnosis of disease. The body has a special system to remove a variety of infectious and toxic materials, one of which is leukocytes or white blood cells. Blood test results can be used as a good parameter and can generally describe the condition of the body such as the examination of the total number of leukocytes (Guyton and Hall, 2011).

There has been no research or data on the total type of white rat *(Rattus norvegicus)* leukocytes induced in DMBA and given serum tadpoles (*Rana catesbeiana).* Based on this, a research needs to be done to find out the effect of tadpoles serum (*Rana catesbeiana*) on total white rat leukocytes (*Rattus norvegicus*) which suffer from cancer induced DMBA.

2 MATERIALS AND METHODS

This study used 20 male white rats (*Rattus norvegicus*) which weighed around 150-250 grams. The experimental animals were then divided into 2 control groups (negative and positive) and 3 treatment groups.

The tools used in this research are weight weighing rats, rat cage, drink container, food container, litter or cage from wood powder, 1 ml disposable syringe with tuberculin needle, 100ml beaker glass, 100 ml measuring cup, analytical scales, filter paper, and camera. The ingredients for the cancer-trigger used in this study are DMBA (7,12-Dimethylbenz-α-anthracene) dissolved in corn oil. DMBA injection (7,12-Dimethylbenz - α-anthracene) was done using 1 ml disposable syringe with tuberculin needle and sterile cotton with 70% alcohol.

Tadpoles blood-collecting (*Rana catesbeiana*) was done using syringe 1 ml disposable with tuberculin needle. Blood was collected using an EDTA tube without anticoagulation. The tube was covered with aluminum foil and centrifuged. Serum dilution was done using PZ or NaCL physiological 0.9% then injected in white rat (*Rattus norvegicus*) using 1 ml disposable syringe with tuberculin needle.

Dilution of DMBA (7,12-Dimethylbenz-α-anthracene) was performed before inducing cancer. Dilution was done using corn oil. Corn oil served as a solvent of DMBA (7,12-Dimethylbenz-α-anthrancene). Dosage for DMBA induction (7,12-Dimethylbenz-α-anthrace) as a trigger for raising cancer cells was 20 mg / kg BW (Cabecas et al, 2014). DMBA induction (7,12-Dimethylbenz-α-anthracene) to induce cancer cells was injected subcutaneously using a 26G size needle. DMBA powder (7,12-Dimethylbenz-α-anthrance) was dissolved in advance with corn oil in order to facilitate the induction process. Comparison of DMBA powder (7,12-Dimethylbenz-α-anthrance) with corn oil is 1 ml of corn oil containing 20 mg DMBA (7.12-Dimethylbenz-α-anthrance). Induction was done for 14 days with duration of twice a week. Subcutaneous induction was performed subcutaneously on the nape of white rats (*Rattus norvegicus*). Cancer observations were performed after the first injection of DMBA (7,12-Dimethylbenz-α-anthrance) by palpation of the injection or nape and skin portions of other white mice (*Rattus norvegicus*). Cancer observation was also carried out by the measurement of diameter and number of nodules that arised. The expected nodule is a cancer nodule, not an abscess nodule. Palpation and measurements were made daily.

The negative control group was not induced with DMBA, whereas the positive control group was induced with 20 mg/kg BW DMBA. All treatment groups were induced by 20 mg/kg BW DMBA. The treatment stage after 14 days was induced with DMBA and after the appearance of skin nodules, the white rats treated group were injected with 1.06 ml tadpoles serum (Mondou dan Kaltenbach, 1979), in P1(100%), P2 (75%) and P3 (25%). Injecting tadpoles serum was done once a day for seven days.

Blood sampling was performed through the heart (Cardiac puncture) using a 2 ml disposable syringe in rats on the 44th day. Blood was then accommodated in an EDTA tube as to not affect the size and shape of the erythrocytes or the shape of the leukocytes (Bijanti *et al.*, 2010). Blood examination was done using Hematology Analyzer HORIBA ABX MICROS 60 instrument and then connected with computer. Blood was homogenized first using Roller-Mixer for 1-2 minutes before checking using Hematology Analyzer.

3 RESULT AND DISCUSSION

Table 1 : Mean of Total Number of Leukocytes in White Rat due to Tadpole Serum (*Rana catesbeiana*) influence.

Treatment	Mean ± SD
K-	12000,00^{a} ± 3814,88
K+	3975,00^{b} ± 2451,36
P1	8650,00^{ab} ± 5470,83
P2	6390,50^{b} ± 3007,18
P3	5590,00^{b} ± 1292,18

Description : Different Superscript in the same

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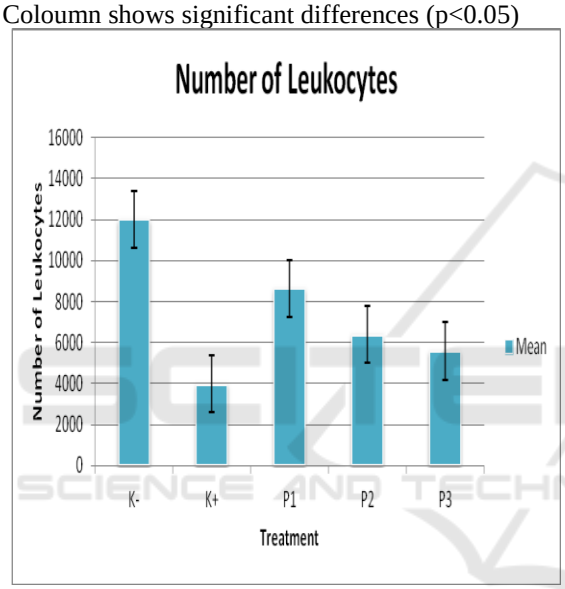

Figure 1: Diagram of White Rat Leukocyte Due to Giving Tadpole Serum (*Rana catesbeiana*).

The K+ group (DMBA 20 mg / kg BB + solvent corn oil) showed the lowest number of 3,975,00^{b} ± 2,451,36 compared with the negative group and all treatment groups, this proves that DMBA can suppress the activity of bone marrow and splenosit so that it can reduce the number of leukocytes especially neutrophils, monocytes and lymphocytes (Akrom *et al.*, 2013)

In P1, it showed the highest increase of total leukocyte number which is 8650,00^{ab} ± 5470,83, then followed by P2 that is 6,390,50^{b} ± 3,007,18, and P3 that is 5,590,00^{b} ± 1,292,18. The increase in total leukocytes in the treatment group 1 to treatment 3 showed that the addition of young tailed frog serum increased the number of cancer-induced white rat leukocytes (*Rattus norvegicus*) using DMBA (Dimethylbenz-α-anthracene). An increase in the number of leukocytes is estimated as a result from an increase in the number of natural killer cells. Tadpoles serum acts as an immunomodulator that stimulates the immune system, such as improving macrophage activity, increasing antibodies and activating natural killer cells. The increase is also associated with the entry of foreign bodies in the body and the response of leukocytes as a mean of body defense (Gigena *et al.*, 2017).

The treatment group showed that higher doses of tadpoles serum in DMBA-induced rats would increase the number of leukocytes in the blood. This result is consistent with the Akrom and Ermawati (2009) study stated that by administering black cumin as immunopreventive at higher doses can increase the number of leukocytes in DMBA-induced Sprague Dawley rats. The higher the dose of the tadpoles serum, the higher the number of leukocytes. It can be said that higher serum doses can inhibit the development of cancer so that the number of leukocytes increases. This illustrates the correlation between the serum content of tadpoles, thyroxine hormones, and increased immune system in animals. With the higher doses, the thyroxine hormone content in tadpole serum is higher, so the ability to increase the immune system is getting bigger.

Table 2 : Average and Standard Deviation of White Rat Leucocyte Types Due to Tadpole Serum (*Rana catesbeiana*) Influence.

Treatment	Lymphocytes (/ mm^3) (X ± SD)	Monocyte (/ mm^3) (X ± SD)	Granulocytes (/ mm^3) (X ± SD)
K-	9900,00^{a} ± 3402,94	600,00^{a} ± 182,57	1500,00^{a} ± 496,65
K+	3275,00^{a} ± 2087,06	250,00^{a} ± 191,48	450,00^{b} ± 208,16
P1	4575,00^{a} ± 4601,72	1050,00^{a} ± 858,29	1225,00^{b} ± 917,87
P2	5058,50^{a} ± 2213,10	793,00^{a} ± 465,25	677,50^{b} ± 384,74
P3	4549,00^{a} ± 1298,10	684,00^{a} ± 138,41	495,50^{b} ± 189,24

Description: Different superscripts in the same column show significant differences (p <0.05)

According to Kusumawati (2004), the normal monocyte value of rats is 0.00 - 0.10 (x 10^3 / mm^3). It can be seen from the average number of monocytes that positive control group has the lowest average monocyte count of all treatments. This is because the positive control of rats were given cancer by DMBA-induced without any serum

treatment, so that DMBA suppresses bone marrow activity resulting in decreased monocyte count (Akrom *et al*., 2013).

Granulocytes are white blood cells characterized by granules in the cytoplasm. Granulocytes such as neutrophils, eosinophils, and basophils are very few in normal circumstances, but when there is an antigen the amount will increase (Fitria and Sarto, 2014). In this study, the average number of granulocytes of the lowest rat in the K+ group was $450{,}00^{b} \pm 208.16$ compared with the K- group and the all-treatment group. This study shows that DMBA induction in rats can decrease the average number of animal granulocytes. In the K- group with the K+, P1, P2, and P3 groups, there was a marked difference ($p < 0.05$). The P1, P2, and P3 groups showed an increase in the number of granulocytes compared to the K- groups, although the statistics showed that the increase was not significantly different ($p > 0.05$).

4 CONCLUSION

The conclusions of this study are:

1. There was an effect of tadpole serum (*Rana catesbeiana*) on the total leucocyte increase of white rats (*Rattus norvegicus*) with DMBA-induced (Dimetylbenz-α-anthracene).
2. There was an effect of tadpole serum (*Rana catesbeiana*) on the increase of white rat type leukocytes (*Rattus norvegicus*) with DMBA-induced (Dimetylbenz-α-anthracene).

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